



Applying Universal HPLC Detection to Measure Proteins, Peptides, and Amino Acids

Introduction

Measurement of biologics is becoming increasingly important to the pharmaceutical industry as many new therapeutics based on proteins or peptides are being brought to market. Analytical techniques to measure ever more sophisticated protein/peptide therapeutics and their formulations are in great demand. Charged Aerosol Detection for HPLC has been shown to be capable of measuring various types of biological molecules in a sensitive, accurate, and comprehensive fashion. Whether measuring biological products themselves, or when working in the process of developing or formulating these molecules, Charged Aerosol Detection can be a technique of great and widespread utility.

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Using proteins and peptides as therapeutics is a relatively new area for the pharmaceutical world. Well-versed in the development, formulation, and production of small molecule therapeutics, many companies still struggle to develop biological products. While the processes used to develop small molecules and biologicals are similar in many respects (potency, stability, formulation, etc.) the sheer complexity of both the biological molecules themselves, and the analytical techniques required to monitor these molecules can make for a daunting analytical challenge. Measurement of many proteins has typically involved a number of different assay techniques, ranging from simple UV measurements, up to sophisticated fluorescence measurement techniques. Pharmaceutical development and QC scientists have utilized a mixture of older technologies (UV, fluorescence, refractive index, and mass spec) in attempts to come up with an HPLC detection scheme that would give the universality, sensitivity, dynamic range, consistent response, and reproducibility necessary for handling the wide range of detection problems with biologicals they face on an on-going basis. Development of a truly universal HPLC detection scheme for proteins/peptides and their formulation ingredients was not possible due to detection technology limitations on HPLC instrumentation (dependence on chromophores for detection, lack of dynamic range, problems with measuring non- or weak UV chromophore molecules existing as impurities or degradants, product intermediates, or formulation components). Couple the problems in accurately monitoring proteins/peptides with the need to measure classic non-UV absorbing molecules as polymers, phospholipids, and sugars often used for excipient packages, and many pharmaceutical development groups working in biologicals struggle with unsolvable analytical problems given their existing analytical capabilities.



A universal HPLC detection technology, charged aerosol detection (CAD[®]), being used to overcome these analytical problems for monitoring and measuring:

- Intact Proteins
- Peptides
- Amino Acids

Protein and Peptide Measurement

Many analytical schemes to measure proteins/peptides are slow, labor-intensive, or serial processes (gel electrophoresis, for example) that require a great deal of expertise to utilize properly. Ideally, having a simple scheme based on technology available in all Pharmaceutical Development and Manufacturing QC groups (HPLC) would be an ideal way to monitor complex proteins. While many proteins have UV chromophores and can be monitored by UV measurement, differences in response factors for different proteins and their degradents can greatly effect the measurement and quantitation of these molecules. Effects of protein size, structure, and solubility can also be problematic. These problems can be exacerbated when studying peptides or their derivatives, especially when dealing with unknown degradents or new peptide structures.

Utilizing CAD, the ability to measure even low levels of these difficult to characterize biological molecules is possible. As seen in Figure 1, the Corona[®] CAD can be utilized to reproducibly measure even low levels of proteins of a wide variety of molecular weights (MWs) and structures. Several proteins of different size, charge, and MW were separated by reverse phase gradient HPLC utilizing a C18 column and an acetonitrile:water gradient. All proteins (and isoforms thereof) were readily detected by charged aerosol detection. Figure 2 illustrates the utilization of the CAD detector for monitoring and measuring the separation of a number of peptides utilizing a reverse phase separation scheme. Once again, the peptides are all readily detected and with very similar response factors, regardless of the differences in chemical structure between the peptides.

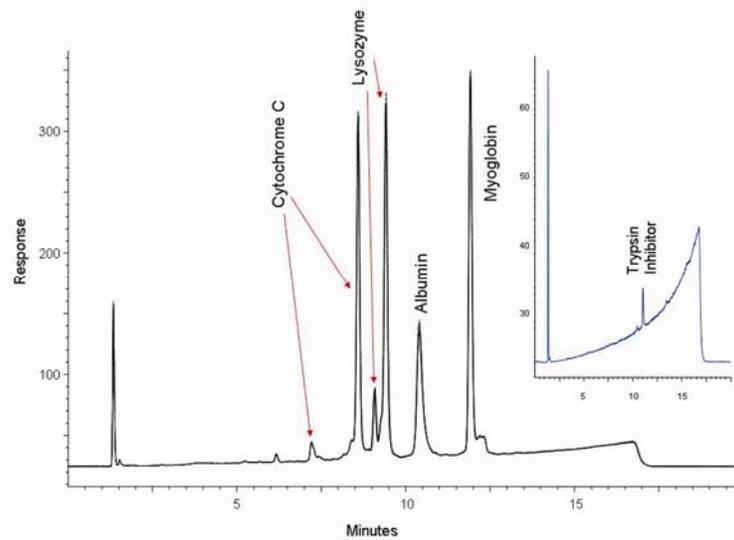


Figure 1. Analysis of Proteins by CAD

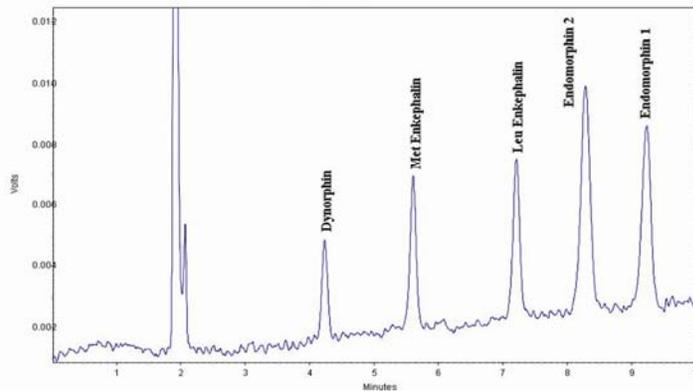


Figure 2. Analysis of Peptides by CAD

Amino Acid Analysis and More

Another key problem area when working with biologicals has been the measurement of amino acids. Sometimes this is needed when working on peptide mapping or sequencing studies of a protein or peptide. Additionally, when producing proteins or peptides recombinantly, measuring individual components of the fermentation broth (amino acids, sugars, etc.) can be important in optimizing and monitoring production levels of a protein of interest. Currently, to measure amino acids, many researchers resort to derivatization of the amino acid by a fluorescent label. However, as seen in Figure 3, CAD is capable of

detecting even underivatized individual amino acids at low ng levels. By using CAD, the complexity and uncertainty inherent in derivatization measurements can be removed from amino acid detection. Some groups utilizing CAD have also been able to measure sugars in fermentation broths or even do oligosaccharide mapping from recombinant proteins with CAD.

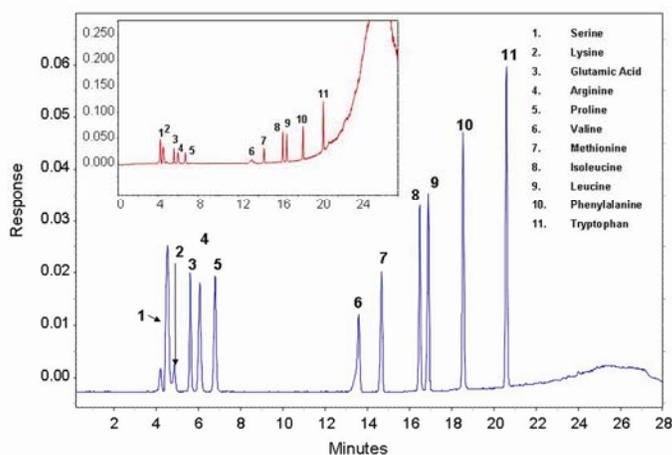


Figure 3. Analysis of Amino Acids by CAD

Conclusion

Charged aerosol detection (CAD) provides universal detection of proteins, peptides, and amino acids. Utilization of CAD allows for the sensitive and accurate detection of these complex biomolecules by HPLC. With CAD, measuring biological therapeutics and even their formulation ingredients by HPLC becomes routine. The combination of the attributes of CAD (universality, consistent response, sensitivity, dynamic range, reproducibility, and ease of use) in a single HPLC detection platform has revolutionized the ability of scientists in pharmaceutical development and manufacturing QC operations for biologicals to replace older existing technologies and use CAD universal detection in key decisions about their samples that can have profound implications for their projects and institutions.